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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/827,854	04/05/2001	Vassilis I. Zannis	07180/004003	6635
21559	7590	04/21/2004	EXAMINER	
CLARK & ELBING LLP 101 FEDERAL STREET BOSTON, MA 02110			NGUYEN, QUANG	
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1636

DATE MAILED: 04/21/2004

Please find below and/or attached an Office communication concerning this application or proceeding.

2/19

Office Action Summary

Application No.

09/827,854

Applicant(s)

ZANNIS ET AL.

Examiner

Quang Nguyen, Ph.D.

Art Unit

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-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --
Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

- 1) ☒ Responsive to communication(s) filed on 22 January 2004.
- 2a) ☐ This action is **FINAL**. 2b) ☒ This action is non-final.
- 3) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

- 4) ☒ Claim(s) 30-47, 50, 51 and 53-78 is/are pending in the application.
- 4a) Of the above claim(s) 32, 35, 38-42 and 45 is/are withdrawn from consideration.
- 5) ☐ Claim(s) _____ is/are allowed.
- 6) ☒ Claim(s) 30, 31, 33, 34, 36, 37, 43, 44, 46, 47, 50, 51 and 53-78 is/are rejected.
- 7) ☐ Claim(s) _____ is/are objected to.
- 8) ☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

- 9) ☐ The specification is objected to by the Examiner.
- 10) ☐ The drawing(s) filed on _____ is/are: a) ☐ accepted or b) ☐ objected to by the Examiner.
Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).
Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).
- 11) ☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

- 12) ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
- a) ☐ All b) ☐ Some * c) ☐ None of:
- 1) ☐ Certified copies of the priority documents have been received.
 - 2) ☐ Certified copies of the priority documents have been received in Application No. _____.
 - 3) ☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

- 1) ☒ Notice of References Cited (PTO-892)
- 2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
- 3) ☒ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08)
Paper No(s)/Mail Date 1/22/04.
- 4) ☐ Interview Summary (PTO-413)
Paper No(s)/Mail Date. _____
- 5) ☐ Notice of Informal Patent Application (PTO-152)
- 6) ☐ Other: _____

DETAILED ACTION

Applicants' amendment filed on 1/22/04 has been entered.

Claims 30-47, 50-51 and 53-78 are pending in the present application.

Claims 32, 35, 38-42, 45 are withdrawn from further consideration because they are drawn to non-elected species.

Accordingly, amended claims 30-31, 33-34, 36-37, 43-44, 46-47, 50-51 and 53-78 are examined on the merits with SEQ ID NO:15 (apoE3) and adenoviral vector as the elected species.

Response to Amendment

The rejections under 35 U.S.C. 102(b) as being anticipated by Tsukamoto et al. (J. Clin. Invest. 100:107-114, 1997) or Kashyap et al. (J. Clin. Invest. 96:1612-1620, 1995) as evidenced by Breslow et al. (J. Biol. Chem. 257:14639-14641, 1982) are withdrawn in light of Applicants' amendment.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 30-31, 33-34, 36-37, 43-44, 46-47, 50-51, 53-55, 57, 59, 61, 72-74 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled

in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. **This is a new ground of rejection.**

Vas-Cath Inc. v. Mahurkar, 19USPQ2d 1111 (Fed. Cir. 1991), clearly states that "applicant must convey with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession of *the invention*. The invention is, for purposes of the 'written description' inquiry, *whatever is now claimed*." *Vas-Cath Inc. v. Mahurkar*, 19USPQ2d at 1117. The specification does not "clearly allow persons of ordinary skill in the art to recognize that [he or she] invented what is claimed." *Vas-Cath Inc. v. Mahurkar*, 19USPQ2d at 1116.

Applicant's invention is drawn to a method of lowering cholesterol in a mammal without inducing hypertriglyceridemia, said method comprising administering to said mammal any nucleic acid encoding a polypeptide having fewer than 299 amino acids, and as long as the polypeptide comprises a region of at least 150 amino acids having at least 80% sequence identity to any mature, native, human apoE polypeptide. Please note that the encoded polypeptide utilized in the methods as claimed may still possess the carboxyl-terminal region of any mature, native, human apoE as long as the polypeptide comprises a region of at least 150 amino acids having at least 80% sequence identity to a mature, native, human apoE polypeptide.

Apart from the disclosure of amino acid sequences of various human apoE isoforms such as apoE4, apoE3, apoE2, apoE1, apoE2* and apoE2** with SEQ ID NOS: 14-19, respectively, and that the amino-terminal 1-185 residues of human apoE4 are sufficient for binding to lipoprotein remnants to an extent that promotes their efficient

clearance in apoE-deficient mice, whereas the carboxyl-terminal 260-299 region of the human apoE4 contributes to hypertriglyceridemia, the specification fails to disclose a sufficient representative number of species for a broad genus of the encoded polypeptide to be utilized in the methods as claimed to lower the total serum cholesterol level without inducing hypertriglyceridemia. The instant specification fails to describe which amino acids to be substituted, deleted or inserted, at which positions and in which combinations such that an encoded polypeptide with fewer than 299 amino acids and comprising a region of at least 150 amino acids having at least 80% sequence identity to any mature, native, human apoE polypeptide, including the elected apoE3 of SEQ ID NO:15, still possesses the desired property, for this instance lowering the total serum cholesterol level without inducing hypertriglyceridemia. Additionally, although the state of the prior art at the effective filing date (4/6/2000) of the present application predominantly suggested that ApoE functioned to decrease cholesterol while increasing triglyceride levels, there were few findings indicated that under certain experimental conditions the infection of ApoE-deficient mice with a recombinant adenovirus capable of expressing a mature, full-length human ApoE resulted in a reduction in the plasma total cholesterol level without an induction of hypertriglyceridemia (Tsukamoto et al., J. Clin. Invest. 100:107-114, 1997; Kashyap et al., J. Clin. Invest. 96:1612-1620, 1995). Thus, it is apparent that at the effective filing date of the present application the biological activity of the apoE proteins with respect to their ability to lower the total serum cholesterol level without inducing hypertriglyceridemia was unpredictable. Moreover, even one year after the effective filing date of the present application,

Applicants still state "The identification of amino acid residues within the carboxyl terminal region of apoE, which mediate the hypertriglyceridemic effect of apoE, **is the subject of ongoing research**" (Kypreos et al., J. Biol. Chem. 276:19778-19786, 2001; page 19785, col. 2, bottom of the first full paragraph). Furthermore, variable isoform-specific effects of apoE polypeptides *in vivo* have also been reported (Yoshida et al., Circulation 104:2820-2825, 2001).

Thus, the claimed invention as a whole is not adequately described if the claims require essential or critical elements which are not adequately described in the specification and which are not conventional in the art as of Applicants' filing date. Possession may be shown by actual reduction to practice, clear depiction of the invention in a detailed drawing, or by describing the invention with sufficient relevant identifying characteristics such that a person skilled in the art would recognize that the inventor had possession of the claimed invention. Pfaff v. Wells Electronics, Inc., 48 USPQ2d 1641, 1646 (1998). The skilled artisan cannot envision the detailed structure of a representative number of species for a broad genus of an encoded polypeptide with fewer than 299 amino acids and comprising a region of at least 150 amino acids having at least 80% sequence identity to any mature, native, human apoE polypeptide, including the elected apoE3 of SEQ ID NO:15, to be utilized in the methods as claimed to lower the total serum cholesterol level without inducing hypertriglyceridemia, and therefore conception is not achieved until reduction to practice has occurred, regardless of the complexity or simplicity of the method. Adequate written description requires more than a mere statement that it is part of the invention and reference to a potential

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method of isolating it. See *Fiers v. Revel*, 25 USPQ2d 1601, 1606 (Fed. Cir. 1993) and *Amgen Inc. v. Chugai Pharmaceutical Co. Ltd.*, 18 USPQ2d 1016 (Fed. Cir. 1991). One cannot describe what one has not conceived. See *Fiddes v. Baird*, 30 USPQ2d 1481, 1483.

Applicant is reminded that *Vas-Cath* makes clear that the written description provision of 35 U.S.C. §112 is severable from its enablement provision (see page 1115).

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

With respect to the elected invention and species, claims 30-31, 33-34, 36-37, 43-44, 46-47, 50-51 and 53-78 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for:

A method of lowering cholesterol in a mammal that lacks an endogenous normally functioning apoE gene, said method comprises administering intravascularly into said mammal a recombinant replication defective adenovirus containing a nucleic acid encoding a polypeptide selected from a group consisting of: the amino acid residues 1-185 of SEQ ID NO:2, the amino acid residues 1-202 of SEQ ID NO:2, the amino acid residues of 1-229 of SEQ ID NO:2 and the amino acid residues of 1-259 of

SEQ ID NO:2, wherein said polypeptide is expressed and the total serum cholesterol level in said mammal is lowered without inducing hypertriglyceridemia,

does not reasonably provide enablement for a method of lowering cholesterol in any mammal without inducing hypertriglyceridemia by administering via any route of delivery or expressing in any tissues in said mammal any nucleic acid molecule, including a recombinant adenovirus containing a nucleic acid encoding a polypeptide having fewer than 299 amino acids, wherein said polypeptide comprises a region of at least 150 amino acids having at least 80% sequence identity to a mature, native, human apoE3 polypeptide. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention commensurate in scope with these claims. **This rejection contains a new ground of rejection.**

The factors to be considered in the determination of an enabling disclosure have been summarized as the quantity of experimentation necessary, the amount of direction or guidance presented, the state of the prior art, the relative skill of those in the art, the predictability or unpredictability of the art and the breadth of the claims. *Ex parte Forman*, (230 USPQ 546 (Bd Pat. Appl & Unt, 1986); *In re Wands*, 858 F.2d 731, 8 USPQ 2d 1400 (Fed. Cir. 1988)).

The instant amended claims are drawn to a method of lowering cholesterol in a mammal without inducing hypertriglyceridemia, said method comprising administering to said mammal a nucleic acid encoding a polypeptide having fewer than 299 amino acids, wherein said polypeptide comprises a region of at least 150 amino acids having at least

80% sequence identity to a mature, native, human apoE polypeptide, and that, when administered to or expressed in a mammal lowers the total serum cholesterol level without inducing hypertriglyceridemia; the same method with various limitations recited in the dependent claims.

The specification teaches by exemplification showing the construction of recombinant adenoviruses expressing apoE4 and various truncated forms of apoE4 (e.g., apoE4-185, apoE4-202, apoE4-229, apoE4-259). In an apoE-deficient mouse model, the recombinant adenoviruses were injected intravenously through the tail vein and the effects of full-length apoE4 and its various truncated forms on cholesterol and triglyceride homeostasis were evaluated. Applicants demonstrated that an insignificant reduction in the mouse cholesterol level and a severely induced hypertriglyceridemia were observed in apoE-deficient mice treated with full-length apoE4-adenovirus, whereas reduced levels of cholesterol without the induction of hypertriglyceridemia were obtained in animals treated with recombinant adenoviruses expressing the aforementioned truncated forms of apoE4. Applicants further demonstrated that overexpression of either full-length apoE3 or apoE4 is sufficient to induce combined hyperlipidemia (high cholesterol and triglyceride levels) in normal C57BL6 mice, whereas an overexpression of apoE4-202 has no detectable effect on triglyceride levels of the C57BL6 mice.

The above evidence has been noted and considered. However, the evidence is not reasonably extrapolated to the instant broadly claimed invention for the following reasons.

(a) The breadth of the claims

The claims encompass a method of lowering cholesterol in any mammal (e.g., a mammal lacking an endogenous normally functioning apoE gene, a mammal lacking an endogenous normally functioning LDL receptor or a mammal having any lipid disorder) without inducing hypertriglyceridemia, said method comprises any route of delivering to said mammal any nucleic acid (e.g., viral or non-viral vector) encoding a polypeptide having fewer than 299 amino acids, wherein said polypeptide comprises a region of at least 150 amino acids having at least 80% sequence identity to any mature, native, human apoE polypeptide; with human apoE3 polypeptide having SEQ ID NO:15 and adenoviral vector as the elected species.

(b) The state and the unpredictability of the art

The nature of the instant claims falls within the realm of gene therapy. At the effective filing date of the present application (4/6/2000), the state of the gene therapy art was and still remains unpredictable particularly for the attainment of desired therapeutic effects, for this instance lowering the total serum cholesterol level without inducing hypertriglyceridemia in any mammal, including a mammal having any lipid disorder, as evidenced by the reviews of Verma et al. (Nature 389:239-242, 1997; IDS), Dang et al. (Clin. Cancer Res. 5:471-474, 1999), Romano et al. (Stem Cells 18:19-39, 2000) and Kawashiri et al. (Curr. Control Trials Cardiovasc. Med. 1:120-127, 2000). Dang et al. stated "Although significant progress has been achieved in our understanding of the limitations of gene therapy by suboptimal vectors, host immunological responses to the vectors, and the lack of long term stable expression,

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the major challenge that limits clinical translation remains in achieving efficient gene delivery to target tissues" (page 474, col. 2, last paragraph). Romano et al. stated "The potential therapeutic applications of gene transfer technology are enormous. However, the effectiveness of gene therapy programs is still questioned" (see abstract), and "Despite the latest progress reported in the area of vector design, research strategies still have to tackle critically important issues, such as further improvement of gene transfer technology, especially for *in vivo* gene delivery applications, regulation and control of the transgene expression post-cell transduction, and a variety of complex safety matters. These three main issues are to some extent intertwined and pose severe limitations on the applications of gene transfer technology in therapy" (page 21, col. 1, first paragraph). In October 2000, Kawashiri et al. still stated "Somatic gene therapy is a viable approach to the therapy of several lipid disorders for which therapies are currently inadequate" and "The next decade is therefore likely to witness several clinical trials of gene therapy for lipid disorders" (see Conclusion section, page 125). Kypreos et al. (FASEB J. 15:1598-1600, 2001) also stated "One major parameter in successful gene therapy approaches is gene dosage and expression levels....The inability of the truncated apoE forms that lack all or part of the carboxyl-terminal 260-299 region to induce hypertriglyceridemia, coupled with their intact ability to clear cholesterol, makes them attractive candidates in future gene therapy applications to correct remnant removal disorders" (page 1600, col. 2, last paragraph). Thus, it is clear that at the effective filing date of the present application gene therapy for the treatment of any lipid disorder was still immature and not routine.

Additionally, at the effective filing date of the present application (4/6/2000) although substantial evidence in the prior art as well as the findings of the present invention suggested or indicated that ApoE functioned **to decrease cholesterol while increasing triglyceride levels** (see references cited on page 6, lines 4-25 of the instant specification), the findings of Tsukamoto et al. (J. Clin. Invest. 100:107-114, 1997; Cited previously) and Kashyap et al. (J. Clin. Invest. 96:1612-1620, 1995) indicated that under their experimental conditions the infection of ApoE-deficient mice with a recombinant adenovirus capable of expressing a mature, full-length human ApoE (299 amino acid residues) resulted in a reduction in the plasma total cholesterol level without an induction of hypertriglyceridemia. Thus, at the effective filing date of the present application it was apparent that the biological activity of the ApoE proteins with respect to their ability to maintain cholesterol and triglyceride homeostasis *in vivo*, at least in ApoE-deficient mice, is unpredictable, let alone in any mammal particularly one with any lipid disorder or disease. Furthermore, at least one year after the filing date of the present application Applicants still state "The identification of amino acid residues within the carboxyl terminal region of apoE, which mediate the hypertriglyceridemic effect of apoE, **is the subject of ongoing research**" (Kypreos et al., J. Biol. Chem. 276:19778-19786, 2001; page 19785, col. 2, bottom of first full paragraph).

The unpredictability of the biological activity of the ApoE proteins to maintain cholesterol and triglyceride homeostasis *in vivo* is further supported by the results of Yoshida et al. (Circulation 104:2820-2825, 2001) that showed that ApoE-deficient mice receiving apoE^{-/-} bone marrow cells that express human apoE3 or apoE2 or

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apoEcys142 have cholesterol levels increasing with age and the cholesterol levels are not affected by apoE expression (see abstract). Interestingly, the lesion in male apoE3 mice was 40% smaller than that of control mice, while the lesion of apoE2 mice was similar to that of control mice and apoEcys142 mice showed an unexpected and significant increase in lesion size. It is further noted that ApoE2 differs from apoE3 by having a cysteine instead of an arginine at residue 158; and apoEcys142 contains 2 amino acid substitutions: an arginine substitution for cysteine at residue 142 and an arginine for cysteine substitution at residue 112.

(c) The amount of direction or guidance presented

Apart from the exemplification using an apoE-deficient mouse model with recombinant adenoviruses expressing apoE4 or one of the truncated apoE variants apoE4-185, apoE4-202, apoE4-229, EpoE4-259, the instant specification fails to provide sufficient guidance for a skilled artisan on how to lower the total serum cholesterol level without inducing hypertriglyceridemia in any mammal using any nucleic acid, including a recombinant adenovirus, encoding a polypeptide having fewer than 299 amino acids, wherein said polypeptide comprises a region of at least 150 amino acids having at least 80% sequence identity to any mature, native, human apoE polypeptide, including apoE3, as broadly claimed. This is because it is uncertain whether the desired therapeutic effects could be obtained in any mammal including a mammal having any lipid disorder or disease by administering a recombinant adenovirus expressing any of the disclosed truncated apoE4, let alone using any nucleic acid encoding a polypeptide having fewer than 299 amino acids as long as it

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comprises a region of at least 150 amino acids having at least 80% sequence identity to any mature, native, human apoE polypeptide. According to a review by Kawashiri et al., ApoE knockout mouse model is not a representative mouse model for any lipid disorder, at best it serves as a model for ApoE deficiency and familial dysbetalipoproteinemia. With regard to the animal model used in gene therapy, Orkin et al. (Report for The Third Meeting of The NIH Investment in Research on Gene Therapy, August, 1995) note that unfortunately, mouse models often do not faithfully mimic the relevant human conditions (Orkin, page 11, second full paragraph), and that animal models are not satisfactory for studying many important disorders, including cystic fibrosis, various cancers, and AIDS. Additionally, Dijk et al. (J. Lipid Res. 40:336-344, 1999, IDS) demonstrated that in LDL receptor-deficient mice both low and high expression of apoE3 via adenovirus-mediated gene transfer **did not result in a reduction of hypercholesterolemia**, and severe hypertriglyceridemia was always induced (see abstract and Fig. 1). Linton et al. (J. Clin. Invest. 101:1726-1736, 1998) also demonstrated that reconstitution of macrophage apoE in apoE(-)/low density lipoprotein receptor LDLR(-) mice **had no effect on serum lipid and lipoprotein concentrations**, although it produces plasma apoE levels up to 16-fold higher than in C57BL/6 controls (see abstract and Table 1). More recently, Yoshida et al. (Circulation 104:2820-2825, 2001) demonstrated that ApoE-deficient mice receiving apoE-/- bone marrow cells that express human apoE3 or apoE2 or apoEcys142 have cholesterol levels increasing with age and **the cholesterol levels are not affected by apoE expression** (see abstract). Thus, in light of the state of the relevant art and particularly the unpredictability of the *in vivo* biological activity of the

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apoE proteins as discussed above, coupled with the lack of sufficient guidance provided by the present application, it would have required undue experimentation for a skilled artisan to make and use the methods as claimed.

With respect to the breadth of claims encompassing the utilization of any nucleic acid encoding a polypeptide having fewer than 299 amino acids as long as it comprises a region of at least 150 amino acids having at least 80% sequence identity to any mature, native, human apoE polypeptide to lower the total serum cholesterol level without inducing hypertriglyceridemia in a treated mammal, the instant specification is not enabled for the full breadth of the claims. This is because apart from the exemplification showing that the amino-terminal 1-185 residues of human apoE4 are sufficient for binding to lipoprotein remnants to an extent that promotes their efficient clearance in apoE-deficient mice, whereas the carboxyl-terminal 260-299 region of human apoE4 contributes to hypertriglyceridemia, the instant specification fails to provide sufficient guidance for a skilled artisan on which modification(s), for example deletion, insertion or substitution, in which combination(s), and at which amino acid residues in any mature, native, human apoE polypeptide, including apoE3 as the elected species, so that the modified polypeptide still possesses the desired properties (lowering the total serum cholesterol level without inducing hypertriglyceridemia in a mammal). As is well recognized in the art, any modification (even a "conservative" substitution) to a critical structural region of a protein is likely to significantly alter its functional properties. Even one year after the effective filing date of the present application Applicants still state "The identification of amino acid residues within the

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carboxyl terminal region of apoE, which mediate the hypertriglyceridemic effect of apoE, **is the subject of ongoing research**" (page 19785, col. 2, bottom of first full paragraph). Please note that the encoded polypeptide in the broad claim may still possess the carboxyl-terminal region of a mature, native, human apoE as long as the polypeptide comprises a region of at least 150 amino acids having at least 80% sequence identity to a mature, native, human apoE polypeptide. Then which modification(s) being carried out to the rest of the polypeptide, so that the polypeptide still possesses the desired properties despite the retention of a carboxyl-terminal region of a mature, native, human apoE polypeptide that is known to contribute to hypertriglyceridemia *in vivo*? Furthermore, there is no evidence of record or in the prior art at the effective filing date of the present application that any truncated apoE polypeptide, including truncated apoE3, that is 184 amino acid residues in length or less is still capable of lowering total serum cholesterol level *in vivo*. Therefore, in light of the unpredictability of the biological activity of the ApoE proteins for lowering the total serum cholesterol level without inducing hypertriglyceridemia *in vivo* as discussed extensively above, and particularly in view of **the variable isoform-specific effects of ApoE** reported by Yoshida et al. in ApoE deficient mice; together with the lack of sufficient guidance provided by the present disclosure it would have required undue experimentation for a skilled artisan to make and use the methods as claimed.

With respect to the issue on **any route of administering any nucleic acid molecule** encoding an apoE3 polypeptide into a mammal, apart from the intravenous delivery of the recombinant adenovirus expressing various truncated apoE4

polypeptides in an apoE deficient mouse model, the instant specification fails to provide sufficient guidance for a skilled artisan on how to obtain an effective level of exogenous plasma apoE3 to yield the desired therapeutic effects without the induction of hypertriglyceridemia in the mammal by delivering the recombinant adenovirus to any non-hepatic tissues (e.g., skin, brain, muscle) or via any route of delivery for liver targeting, let alone for any nucleic acid molecule (e.g., viral and/or non-viral vector). Vector targeting *in vivo* to desired tissues or organs (for this instance to the liver) continues to be unpredictable and inefficient. This is supported by numerous teachings available in the art as well as the aforementioned review articles (see Dang et al., Verma et al., Romano et al.). Moreover, Athanasopoulos et al. (Human Molecular Genetics 9:2545-2551, 2000) have shown that intramuscular plasmid injection in apoE^{-/-} mice with plasmid vectors expressing allelic human apoE2 or apoE3 isoforms **did not result in any reduction of plasma cholesterol nor in plasma triglycerides** compared to control injected mice (see Table 1, and abstract). Thus, with the lack of sufficient guidance provided by the present disclosure, it would have required undue experimentation for a skilled artisan in the art to make and use the methods as claimed.

As set forth in *In re Fisher*, 166 USPQ 18 (CCPA 1970), compliance with 35 USC

112, first paragraph requires:

That scope of claims must bear a reasonable correlation to scope of enablement provided by specification to persons of ordinary skill in the art; in cases involving predictable factors, such as mechanical or electrical elements, a single embodiment provides broad enablement in the sense that, once imagined, other embodiments can be made without difficulty and their performance characteristics predicted by resort to known scientific laws; in cases involving unpredictable factors, such as most chemical reactions and physiological activity, scope of enablement varies inversely with degree of unpredictability of factors involved.

Accordingly, due to the lack of sufficient guidance provided by the specification regarding to the issues discussed above, the unpredictability of the gene therapy as well as the relevant art on the biological activity of apoE protein in lowering the total serum cholesterol level without inducing hypertriglyceridemia, and the breadth of the instant claims, it would have required undue experimentation for one skilled in the art to make and use the instant broadly claimed invention.

Response to Arguments

Applicants' arguments related in part to the above rejection in the Amendment filed on 1/22/04 (pages 10-16) have been fully considered, but they are not found persuasive for the following reasons.

1. With respect to the general unpredictability of the art, Applicants argued that none of the references cited by the Examiner suggest that gene therapy does not work or is completely unpredictable. Instead, the references merely suggest that gene therapy has not been optimized sufficiently for certain therapeutic ends, and that optimization of gene therapy requires no more than routine experimentation.

Dang et al. state "This workshop reviewed some recent advances in gene delivery, gene expression, immune manipulation, and the development of molecular targets and stressed that all of these fields will need further advancement **to make gene therapy a reality**" (page 471, col. 1, bottom of first paragraph). Romano et al. state "The potential therapeutic applications of gene transfer technology are enormous. However, the effectiveness of gene therapy programs is still questioned...[d]espite the

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latest significant achievements reported in vector design, **it is not possible to predict to what extent gene therapeutic interventions will be effective in patients, and in what time frame**" (see abstract). Kawashiri et al. state "Somatic gene therapy is a viable approach to the therapy of several lipid disorders for which therapies are currently inadequate" and "The next decade is therefore likely to witness several clinical trials of gene therapy for lipid disorders" (see Conclusion section, page 125). All of these statements do not support Applicants' arguments that the attainment of therapeutic effects via gene therapy was predictable and routine at the effective filing date of the present application.

2. With respect to the issue on duration of gene expression, Applicants argue that unlike many gene therapy trials in the prior art, Applicants' method may not require prolonged periods of transgene expression, and that the temporary expression of ApoE fragments may be sufficient to provide immediate reduction of plasma cholesterol; a condition that may be subsequently maintained by existing drug therapies, dietary modification, or re-injection of a vector of the invention.

Applicants' arguments are moot with respect to the scope of enablement given above.

3. With respect to the cited reference of Dijk et al. (J. Lipid Res. 40:336-344, 1999), Applicants argue that the teachings of Dijk et al. are not inconsistent with Applicants' data with respect to the hypertriglyceridemia-inducing effects of ApoE overexpression.

It is noted that the reference of Dijk et al. was cited to demonstrate overexpression of exogenous apoE3 does not necessarily lead to a lower level of total serum cholesterol in LDL receptor-deficient mice, given the fact that hypertriglyceridemia was also expected because of the utilization of a full-length apoE3.

4. With respect to tissue targeting of the vector, Applicants direct the Examiner's attention to two post-filing arts of Chen et al. (Mol. Ther. 2:256-261, 2000) and Oka et al. (Circulation 103:1274-1281, 2000) demonstrating that transgene expression in the liver was detected for at least six months following infection using adenoviral vectors. Additionally, Applicants argue that tissue-specific expression of the apoE fragment is required neither for a successful clinical outcome nor in the presently claimed invention, therefore it is an inappropriate basis to support for the lack of enablement rejection.

It is noted that both post-filing arts teach intravenous or intravascular administration of the recombinant vectors into treated mice, and not by any route of delivery or at any site. Furthermore, it should also be noted that most apoE in plasma derived from the liver, and that the majority of recombinant adenoviral vectors that are administered by intravenous or intravascular route will be sequestered in the liver. Furthermore, Athanasopoulos et al. (Human Molecular Genetics 9:2545-2551, 2000) demonstrated clearly that intramuscular plasmid injection in apoE^{-/-} mice with plasmid vectors expressing allelic human apoE2 or apoE3 isoforms **did not result in any reduction of plasma cholesterol nor in plasma triglycerides** compared to control injected mice (see Table 1, and abstract).

Accordingly, claims 30-31, 33-34, 36-37, 43-44, 46-47, 50-51 and 53-78 are rejected for the reasons set forth above.

The following is a quotation of the second paragraph of 35 U.S.C. 112:

The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 30-31, 33-34, 36-37, 43-44, 46-47, 53-55, 57, 59, 61 and 72-74 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. **This is a new ground of rejection necessitated by Applicants' amendment.**

In amended claim 30 and its dependent claims, the phrase "having at least 80% sequence identity to the corresponding region of a mature, native, human apoE polypeptide" is unclear, and therefore it renders the claims indefinite. This is because which region of a mature, native, human apoE polypeptide is or is not the corresponding region? The metes and bounds of the claims are not clearly determined.

New claim 73 is incomplete. Therefore, the claim is indefinite because the metes and bounds of the claim are not clearly determined.

Conclusions

No claims are allowed.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's mentor, David Guzo, Ph.D., may be reached at (571) 272-0767, or SPE, Irem Yucel, Ph.D., at (571) 272-0781.

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To aid in correlating any papers for this application, all further correspondence regarding this application should be directed to Group Art Unit 1636; Central Fax No. (703) 872-9306.

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